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Mechanisms of Ageing and Development 127 (2006) 444–450
mechanisms of ageing and development
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Methionine flux to transsulfuration is enhanced in the long living Ames dwarf mouse

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Accepted 5 January 2006
Available online 6 March 2006

Abstract

Long-lived Ames dwarf mice lack growth hormone, prolactin, and thyroid stimulating hormone. Additionally the dwarf mice have enzyme activities and levels that combat oxidative stress more efficiently than those of normal mice. We have shown that methionine metabolism in Ames mice is markedly different than in their wild type littermates. In our previous work we hypothesized that the flux of methionine to the transsulfuration pathway is enhanced in the dwarf mice. The current study was designed to determine whether the flux of methionine to the transsulfuration pathway is increased. We did this by injecting either L-[methyl-^{3}H]-methionine or L-[^{35}S]-methionine into dwarf or normal mice and then determined retained label (in form of *S*-adenosylmethionine) 45 min later. The amount of retained hepatic ^{3}H and ^{35}S label was significantly reduced in the dwarf mice; at 45 min the specific radioactivity of SAM (pCi/nmol SAM) was 56% lower ($p < 0.05$) for ^{3}H-label and 64% lower ($p < 0.005$) for ^{35}S-label in dwarf than wild type mice. Retention of ^{35}S was significantly lower in the brain (37%, $p < 0.04$) and kidney (47%, $p < 0.02$) of the dwarf compared to wild type mice; there was no statistical difference in retained ^{3}H-label in either brain or kidney. This suggests that both the methyl-moiety and the carbon chain of methionine are lost much faster in the dwarf compared to the wild type mouse, implying that both transmethylation in the liver and transsulfuration in the liver, brain, and kidney are increased in the dwarf mice. As further support, we determined by real-time RT PCR the expression of methionine metabolism genes in livers of mice. Compared to wild type, the Ames dwarf had increased expression of methionine adenosyltransferase 1a (2.3-fold, $p = 0.013$), glycine *N*-methyltransferase (3.8-fold, $p = 0.023$), betaine homocysteine methyltransferase (5.5-fold, $p = 0.0006$), *S*-adenosylhomocysteine hydrolase (3.8-fold, $p = 0.0005$), and cystathionase (2.6-fold; tended to be increased, $p = 0.055$). Methionine synthase expression was significantly decreased in dwarf compared to wild type (0.48-fold, $p = 0.023$). These results confirm that the flux of methionine to transsulfuration is enhanced in the Ames dwarf. This, along with data from previous studies support the hypothesis that altered methionine metabolism plays a significant role in the oxidative defense of the dwarf mouse and that the mechanism for the enhanced oxidative defense may be through altered GSH metabolism as a result of the distinctive methionine metabolism. Published by Elsevier Ireland Ltd.

Keywords: Ames dwarf; Methionine metabolism; Real-time RT PCR; Tracer

1. Introduction

Ames dwarf mice live 50–64% longer than their wild type littermates (males and females, respectively) (Brown-Borg et al., 2005) and these mice have a delayed occurrence and reduced incidence of presumably fatal neoplastic disease compared with their normal siblings (Ikeno et al., 2003). Ames dwarf mice have a homozygous recessive mutation at the Prop-1 locus, which leads to a lack of differentiation of somatotrophic, lactotrophic, and thyrotrophic pituitary cells.

Abbreviations: Ahcy, *S*-adenosylhomocysteine hydrolase; Bhmt, betaine homocysteine methyltransferase; Cbs, cystathionine β-synthase; Cth, cystathionase; Gnmt, glycine *N*-methyltransferase; GH, growth hormone; Mat, methionine adenosyltransferase (*S*-adenosylmethionine synthase; methionine activating enzyme); Mtr, methionine synthase (MS); Mthfr, methylenetetrahydrofolate reductase; SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; THF, tetrahydrofolate

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0047-6374/$ – see front matter. Published by Elsevier Ireland Ltd.
doi:10.1016/j.mad.2006.01.001

As a result, these mice lack growth hormone (GH), prolactin, and thyroid stimulating hormone (Hauck et al., 2001).

Recent studies by Brown-Borg (Brown-Borg et al., 1999, 2001; Brown-Borg and Rakoczy, 2000) have focused on the relevance of reduced GH signaling to longevity and lower oxidative stress in the dwarf mice. Growth hormone can also affect other metabolic pathways including methionine metabolism (Fig. 1). For example, glycine *N*-methyltransferase (Gnmt), which plays a crucial role in the regulation of tissue concentrations of *S*-adenosylmethionine (SAM) and *S*-adenosylhomocysteine (SAH), is regulated by GH (Aida et al., 1997). Also, pulsatile GH secretion decreases *S*-adenosylhomocysteine hydrolase (Ahcy) in rat liver (Oscarsson et al., 2001). Thus, the hormonal changes found in the Ames dwarf mouse may affect methionine metabolism. Therefore, in a previous study our objective was to determine whether or not methionine metabolism is indeed altered and if so, whether this may help explain the extended survival of these mice. We found that the long-lived Ames dwarf mouse appears to have an enhanced methionine metabolism. The specific activities of methionine adenosyltransferase (Mat), Gnmt, cystathionine β-synthase (Cbs), and cystathionase (Cth) are significantly increased in liver of the dwarf mice while the hepatic concentration of SAM is decreased and that of SAH increased. In our previous work, we hypothesized that the flux of methionine to the transsulfuration pathway is enhanced in the dwarf mice. To test that hypothesis we conducted an isotope study to follow the metabolism of methionine. This paper presents the findings of a tracer study in which L-[methyl-^{3}H]-methionine or L-[^{35}S]-methionine was injected intraperitoneally into normal or dwarf mice. Exactly 45 min after the injections, the mice were killed and liver, brain, and kidney were obtained for ^{3}H-SAM and ^{35}S-SAM analysis. Previous work showed that in the rodent liver the specific activity of the SAM pool is likely to be identical to that of the methionine pool because of the very rapid turnover rate of the SAM pool (Hoffmann, 1980; Engstrom and Benevenga, 1987; Eloranta et al., 1990). Therefore, based on the amount of ^{3}H or ^{35}S labeled-SAM remaining at 45 min, one can estimate the relative differences of flow of methionine through methionine recycling and the transsulfuration pathway in the dwarf versus the wild type mouse. Also, to further study the mechanism of the hypothesized increase in flux of methionine through the transsulfuration pathway, we present data on the mRNA abundance of enzymes involved in methionine metabolism.

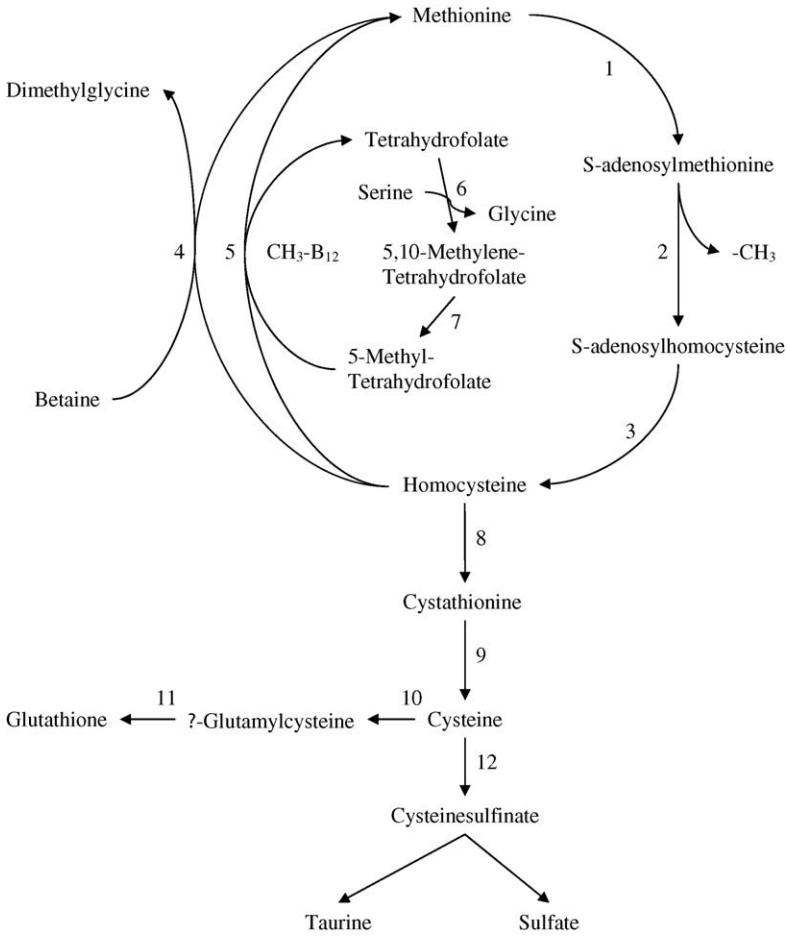

Fig. 1. Methionine metabolism. Enzymes: 1, Mat (methionine adenosyltransferase); 2, SAM-dependent transmethylases including glycine *N*-methyltransferase (Gnmt); 3, *S*-adenosylhomocysteine hydrolase (Ahcy); 4, betaine homocysteine methyltransferase (Bhmt); 5, methionine synthase (Mtr); 6, serine hydroxymethylase; 7, methylenetetrahydrofolate reductase (Mthfr); 8, cystathionine β-synthase (Cbs); 9, cystathionase (Cth); 10, γ-glutamylcysteine synthetase; 11, glutathione synthetase; 12, cysteine dioxygenase.

2. Methods and materials

2.1. Animals

Ames dwarf and age-matched wild type mice were maintained at the University of North Dakota (UND) vivarium facilities under controlled conditions of photoperiod (12 h light:12 h dark) and temperature (22 ± 1 °C) with free access to food (Laboratory Rodent Diet, PMI Nutrition Intl., St. Louis, MO) and water. The Ames dwarf (df/df) mice used in this study were derived from a closed colony with heterogeneous background (over 20 years). Dwarf mice were generated by mating either homozygous (df/df) or heterozygous (df/+) dwarf males with carrier females (df/+). Ames dwarf mice are maintained under standard laboratory conditions. All procedures involving animals were reviewed and approved by the UND Institutional Animal Care and Use Committee in accordance with the NIH guidelines for the care and use of laboratory animals.

2.2. Methionine cycling

The method of Eloranta et al. (1990) was used to estimate the quantitative significance of methionine salvage pathways and transsulfuration in the Ames dwarf versus wild type controls. On a per gram body weight, non-fasted male mice were injected intraperitoneally with either 0.1 μCi L-[methyl-^{3}H]-methionine (83 Ci/mmol; Amersham Biosciences, Piscataway, NJ) or 0.1 μC L-[^{35}S]-methionine (>1000 Ci/mmol; Amersham Biosciences) in 0.9% NaCl. Unlabeled L-methionine was added to each isotope such that 0.05 nmol methionine was injected on a per gram body weight. In a study with rats, Eloranta et al. (1990) injected (as a tracer dose of L-[2-^{14}C]-methionine) approximately 0.1 nmol methionine/g body weight. Based on highest incorporation of label into liver they then determined that if it were totally methionine, it would correspond to less than 2% of the hepatic methionine concentration. Thus, the dose used in our study (0.05 nmol methionine/g body weight) most likely had no effect on tissue methionine concentration. All mice were 17–18-month-old. For L-[methyl-^{3}H]-methionine, four dwarf and four wild type mice were used. For L-[^{35}S]-methionine, four dwarf and five wild type mice were used. The mice were injected and killed in chronological order such that all animals were killed exactly 45 min after injection. Because of the limited number of 17–18-month-old mice available, we decided to use one time point (45 min) in our study. This precluded us from determining the half-life of SAM (either ^{35}S-SAM or ^{3}H-methyl-SAM) in liver, brain, or kidney. However, it still allowed comparison of retention of SAM between the dwarf and wild type mice. The 45 min time was selected as a point where metabolism of labeled methionine would yield sufficient amounts of correspondingly labeled SAM (Eloranta et al., 1990, unpublished observations). Mice were killed by decapitation. Liver, brain, and both kidneys were rapidly excised, weighed, and frozen in liquid nitrogen and stored at −80 °C until time of analysis (within 7 days). Tissues were weighed and homogenized in 3 volumes H_2O (ratio of 1 g tissue to 3 mL H_2O). The contents were transferred to a centrifuge tube and a volume of trichloroacetic acid (20%, w/v) equivalent to the volume of H_2O was added and the tubes were thoroughly mixed. After centrifugation the supernatant was extracted three times with an equal volume of diethylether. The supernatant was then applied to a Cellex-P (H^+) column (1 cm × 7 cm) and washed successively with 1, 10, 50, and 500 mM HCl (Eloranta et al., 1976). *S*-Adenosylmethionine was eluted by 500 mM HCl; this fraction was collected. The concentration of SAM in this fraction was estimated by measuring absorbance at 257 nm and using a molar extinction coefficient of 15,000 L mol/cm (Eloranta et al., 1976). The concentration and purity of SAM in the fraction were also determined by HPLC using the method of Bottiglieri (1990) A portion of the 500 mM HCl fraction was added to Aquasol-2 (Perkin-Elmer, Boston, MA) and counted for either ^{3}H or ^{35}S by liquid scintillation counting.

2.3. Real-time RT PCR

During the animal kill, prior to freezing the liver in liquid nitrogen, a piece of liver was quickly excised and placed in RNAlater (Ambion, Austin, TX) and then stored at −20 °C until RNA extraction. Total RNA was extracted by using the NucleoSpin RNA II Kit (BD Biosciences, Palo Alto, CA). The primers used for the different genes studied are shown in Table 1.

Table 1
Primers

Gene[a]	GenBank accession number	Primer
Mat1a	NM_133653	F: 5′-CTG AGG CGC TCT GGT GTC-3′ R: 5′-TCC TGC ATG TAC TGA ACT GTT ACC-3′
Mat2a	NM_145569	F: 5′-CCG AGT CTG TAG GGG AAG GT-3′ R: 5′-GGT CTT GTT GAA GGT GTG CAT-3′
Gnmt	NM_010321	F: 5′-GCT GGA CGT AGC CTG TGG-3′ R: 5′-CAC GCT CAT CAC GCT GAA-3′
Ahcy	NM_016661	F: 5′-CTG TTG GGG TTC ACT TCC TG-3′ R: 5′-ACA TTC AGC TTG CCC AGG T-3′
Bhmt	NM_016668	F: 5′-ACG TGG ACT TCC TCA TTG CAG AGT-3′ R: 5′-TGC TAC GGG CTT ACC AGA TGC TTT-3′
Mtr	XM_138431	F: 5′-GCA GAT GTG GCC AGA AAA G-3′ R: 5′-GCC ACA AAC CTC TTG ACT CC-3′
Mthfr	NM_010840	F: 5′-AGCTTGAAGCCACCTGGACTGTAT-3′ R: 5′-AGACTAGCGTTGCTGGGTTTCAGA-3′
Cth	NM_145953	F: 5′-GAG TCT GGC TGA GCT TCC A-3′ R: 5′-CGA GGG TAG CTC TGT CCT TC-3′
Cbs	NM_144855	F: 5′-CGC ACA GGA AGG ACT GCT A-3′ R: 5′-AGC CTT CAC AGC CAC AGC-3′
Rn18s	X00686	F: 5′-AAA TCA GTT ATG GTT CCT TTG GTC-3′ R: 5′-GCT CTA GAA TTA CCA CAG TTA TCC AA-3′

[a] LocusLink official symbol followed by official gene name. Mat1a: methionine adenosyltransferase I, alpha; Mat2a: methionine adenosyltransferase II, alpha; Gnmt: glycine *N*-methyltransferase; Ahcy: *S*-adenosylhomocysteine hydrolase; Bhmt: betaine-homocysteine methyltransferase; Mtr: 5-methyltetrahydrofolate-homocysteine methyltransferase; Mthfr: 5,10-methylenetetrahydrofolate reductase; Cth: cystathionase (cystathionine γ-lyase); Cbs: cystathionine β-synthase; Rn18s: 18S RNA.

Real-time RT PCR was done using the QuantiTect SYBR Green RT-PCR Kit (Qiagen, Valencia, CA) and a SmartCycler (Cepheid, Sunnyvale, CA) instrument. Gene expression was quantitated by using the comparative C_T method (Heid et al., 1996). C_T, the threshold cycle, is the number of cycles it takes for a sample to reach the level where the rate of amplification is the greatest during the exponential phase. ΔC_T was obtained for each sample/gene by the following calculation: $\Delta C_T = C_{T,X} - C_{T,R}$, where $C_{T,X}$ is the threshold number for target gene amplification and $C_{T,R}$ is the threshold number for reference gene (Rn18s) amplification. For the comparative C_T method to be valid, replication efficiencies (slope of the calibration curves) of the target gene and that of the reference gene must be approximately equal. For the primers listed in Table 1, replication efficiencies of each target amplifications were equal to the efficiency of the reference amplification (Rn18s) (data not shown).

The amount of target (in the dwarf mice), normalized to an endogenous reference (Rn18s) and relative to the control group (wild type mice), is given by the formula $2^{-\Delta\Delta C_T}$; $\Delta\Delta C_T = \Delta C_{T,q} - \Delta C_{T,cb}$, where $\Delta C_{T,q} = \Delta C_T$ for an individual sample and $\Delta C_{T,cb}$ = mean ΔC_T for control group. Thus, the value of $2^{-\Delta\Delta C_T}$ for the amount of target in the wild type (control group) was 1. For the amount of target in the dwarf mice, a 1-fold change indicates no change, greater than 1-fold change indicates up-regulation, and less than 1-fold change indicates down-regulation.

2.4. Statistics

For real-time RT PCR the *t*-test was used to compare the ΔC_T values; $p < 0.05$ was considered significant. For tracer data, the *t*-test for unequal variances was used; $p < 0.05$ was considered significant.

3. Results

The amount of retained hepatic ^{3}H and ^{35}S label, as measured in SAM, 45 min after injection of L-[methyl-^{3}H]-methionine or L-[^{35}S]-methionine, respectively, was significantly reduced in the dwarf mice. At 45 min the specific radioactivity of SAM (pCi/nmol SAM) was 56% lower ($p < 0.05$) for ^{3}H-label and 64% lower ($p < 0.005$) for ^{35}S-label in dwarf than wild type mice (Fig. 2).

Figs. 3 and 4 show that ^{35}S retention (specific radioactivity of SAM) was significantly lower in the brain (37%, $p < 0.04$) and kidney (47%, $p < 0.02$) of the dwarf compared to wild type mice. There was no statistical difference in retained ^{3}H-label in either brain or kidney when comparing dwarf to wild type mice.

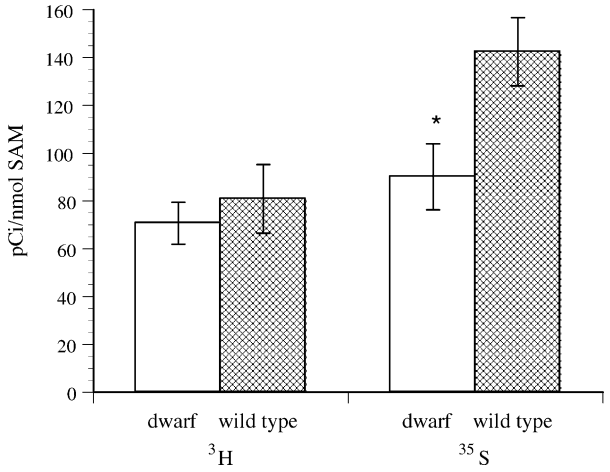

Fig. 3. Specific radioactivity of brain *S*-adenosylmethionine (pCi/nmol) 45 min after injection of either L-[methyl-H]-methionine (left bars, $N = 4$ for dwarf and 4 for wild type) or L-[^{35}S]-methionine (right bars, $N = 4$ for dwarf and 5 for wild type). Means ± S.E.M. $^*p < 0.04$.

Fig. 5 shows the expression of methionine metabolism genes in livers of mice as determined by real-time RT PCR. Data are presented as fold change mRNA from dwarf normalized to wild type (=1). Increased expression of Mat1a (2.3-fold, $p = 0.013$), Gnmt (3.8-fold, $p = 0.023$), Bhmt (5.5-fold, $p = 0.0006$), Ahcy (3.8-fold, $p = 0.0005$), and Cth (2.6-fold; tended to be increased, $p = 0.055$) was observed in dwarf compared to wild type mice. Mtr expression was significantly decreased in dwarf compared to wild type (0.48-fold, $p = 0.023$). No significant changes were seen in expression of Mat2a, Cbs, and Mthfr.

4. Discussion

The results of the isotope portion of this study show that the flux of methionine through the transsulfuration pathway is

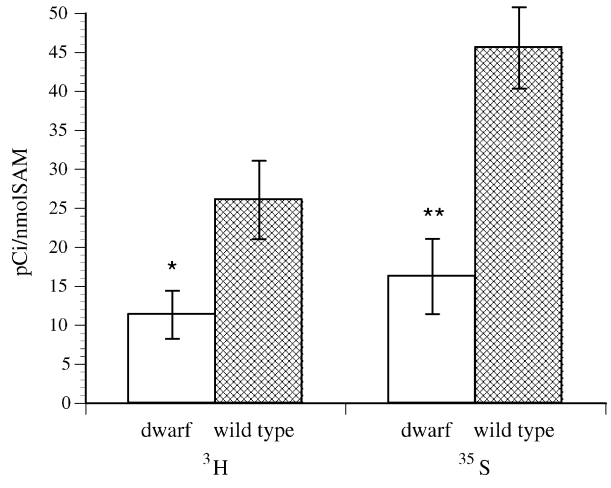

Fig. 2. Specific radioactivity of liver *S*-adenosylmethionine (pCi/nmol) 45 min after injection of either L-[methyl-^{3}H]-methionine (left bars, $N = 4$ for dwarf and 4 for wild type) or L-[^{35}S]-methionine (right bars, $N = 4$ for dwarf and 5 for wild type). Means ± S.E.M. $^*p < 0.05$; $^{**}p < 0.005$.

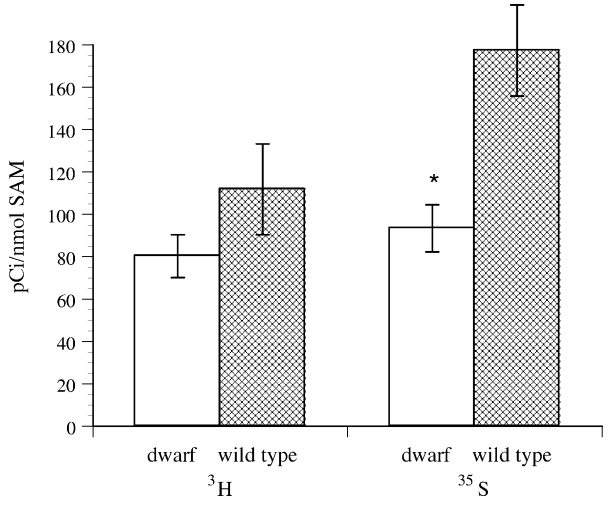

Fig. 4. Specific radioactivity of kidney *S*-adenosylmethionine (pCi/nmol) 45 min after injection of either L-[methyl-^{3}H]-methionine (left bars, $N = 4$ for dwarf and 4 for wild type) or L-[^{35}S]-methionine (right bars, $N = 4$ for dwarf and 5 for wild type). Means ± S.E.M. $^*p < 0.02$.

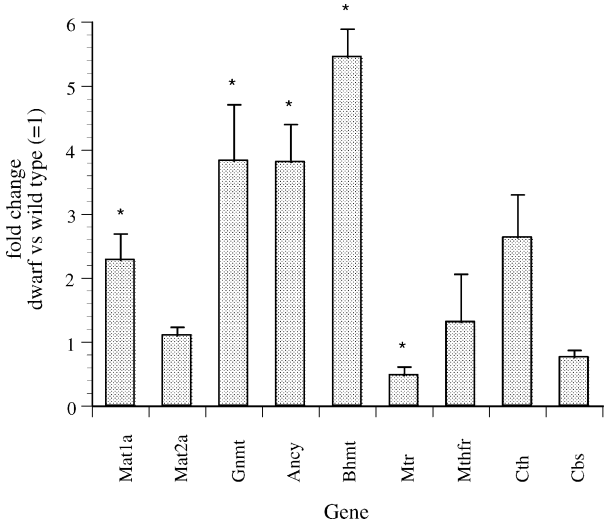

Fig. 5. Expression of methionine metabolism genes in livers of mice; fold change mRNA from dwarf normalized to wild type (=1). The fold change ($2^{-\Delta\Delta C_T}$) was calculated as indicated in the text. *Statistically significant ($p < 0.05$) vs. wild type (fold change = 1); *t*-test compares ΔC_T values. Data are means ± S.E.M. Individual *p*-values—Mat1a: methionine adenosyltransferase I, alpha ($p < 0.013$); Mat2a: methionine adenosyltransferase II, alpha (NS); Gnmt: glycine *N*-methyltransferase ($p < 0.024$); Ahcy: *S*-adenosylhomocysteine hydrolase ($p < 0.0005$); Bhmt: betaine-homocysteine methyltransferase ($p < 0.0006$); Mtr: 5-methyltetrahydrofolate-homocysteine methyltransferase ($p < 0.024$); Mthfr: 5,10-methylenetetrahydrofolate reductase (NS); Cth: cystathionase (cystathionine γ-lyase) ($p < 0.055$); Cbs: cystathionine β-synthase (NS). NS, not significant.

increased in the Ames dwarf. The isotope study clearly shows that retention of both ^{3}H-methyl-SAM and ^{35}S-SAM were significantly decreased (56 and 64%, respectively) in liver of the dwarf mice compared to the wild type. This suggests that both the methyl-moiety and the carbon chain of methionine are lost much faster in the dwarf compared to the wild type mouse, implying that both transmethylation and transsulfuration are increased in the dwarf mice.

In brain and kidney, ^{3}H-methyl-SAM retention was similar in the dwarf and wild type mice. However, ^{35}S-SAM was decreased in dwarf compared to wild type (36% in brain and 47% in kidney). This indicates that transmethylation was similar but that transsulfuration was increased in the brain and kidney of dwarf mice compared to wild type.

The real-time data for liver mRNA support the findings with the isotopes and that of our previous hepatic enzyme work (Uthus and Brown-Borg, 2003). Liver mRNA expression was significantly elevated in the dwarf mouse for enzymes involved in transsulfuration (Mat1a, Gnmt, Achy, and Bhmt) and decreased for Mtr. We found no change with Mat2a and Mthfr.

The changes in enzyme activity and/or mRNA expression explain the increased transmethylation and increased flux of methionine to the transsulfuration pathway. The enzymes that are involved in methionine metabolism (Fig. 1) can be grouped by their kinetic characteristics as either methionine-conserving enzymes (i.e., methionine recycling; MatI, MatII, Ahcy, Bhmt, Mtr, and Mthfr) or methionine-catabolizing enzymes (i.e., transsulfuration; MatIII, Cbs, Cth, and Gnmt) (Finkelstein, 2000). The methionine-conserving enzymes have low K_m values and tend to be inhibited by their products; the methionine-catabolizing enzymes have relatively high K_m values and may be affected positively by methionine metabolites (Finkelstein, 2000). In studies with rats, Finkelstein (2000) found that the hepatic content of the transsulfuration (methionine-catabolizing) enzymes increased with increased dietary methionine or protein. The diet used in our studies is adequate, not luxuriant, in methionine and cysteine providing 0.76% methionine + cysteine. Further, it has been shown that the Ames dwarf mouse does not self-restrict its caloric intake but instead actually consumes greater calories per gram body weight than wild type mice (Mattison et al., 2000). Other work has shown that SAM serves as a metabolic switch such that high concentrations of SAM facilitate transsulfuration and limit methionine recycling (Finkelstein, 2000; Martinov et al., 2000). However, in the Ames dwarf, the dietary sulfur amino acid content and/or liver SAM concentration suggest that methionine recycling should be enhanced at the expense of transsulfuration. This is not the case as the flux of methionine through the transsulfuration pathway is greatly enhanced in the Ames dwarf mouse. Thus, there appear to be other regulatory factors in the Ames dwarf mouse that result in a rapid loss of the methyl moiety of methionine with concurrent loss of the carbon backbone via transsulfuration.

A prime candidate for this regulation is GH, which is lacking in the Ames dwarf mouse. GH has been shown to affect both Mat and Gnmt (Aida et al., 1997; Oscarsson et al., 2001). Methionine is converted to SAM by the enzyme Mat. Oscarsson et al. (2001) showed that Mat activity is decreased by pulsatile GH secretion in rat liver. In mammals, two genes, Mat1a and Mat2a, encode for the enzyme. Mat1a is expressed only in the liver as two isozymes (Mat III and Mat I). Mat2a is widely expressed and encodes for Mat II (Carretero et al., 2001; Lu et al., 2001). However, the assay we used in our original paper did not differentiate between Mat I and III or, if expressed in the mouse livers, Mat II. Our research showed that liver Mat was increased 205% in the dwarf compared to wild type mouse and that GH administration to dwarf mice results in an approximate 40% suppression of Mat activity (Uthus and Brown-Borg, 2003; Brown-Borg et al., 2005). We now show that mRNA expression of Mat1a was increased 2.3-fold; Mat 2a was not different between dwarf and wild type. The increase in enzyme activity/mRNA expression indicates that methionine will readily be converted to SAM in the Ames dwarf.

The SAM produced will readily loose its methyl group because Gnmt enzyme activity is increased 91% (mRNA is increased 3.8-fold) in the dwarf mouse. Gnmt is an enzyme important in the regulation of tissue concentrations of SAM and hence SAH (Cook and Wagner, 1984; Wagner et al., 1985; Loehrer et al., 1996; Aida et al., 1997; Ogawa et al., 1998). This regulation can take place through folate or by SAM itself. In a normal animal, when SAM is low (e.g., low dietary methionine) Gnmt is inhibited thereby conserving SAM for important biological methylation reactions. However, GH decreases the expression and activity of Gnmt (Aida et al., 1997). Because Gnmt is a major enzyme in liver making up between 0.5 and 1% of the soluble protein in rat liver cytosol (Wagner et al., 1985), it

is likely that the changes seen in Gnmt (91% increase in activity and 3.8-fold increase in mRNA) are of physiological significance. In addition, we have shown that GH administration to dwarf mice results in an approximate 44% decrease in activity of hepatic Gnmt (Brown-Borg et al., 2005). Thus, in the Ames dwarf mouse which lacks GH, the concomitant increase in Gnmt activity and expression results in a decrease in the pool size of SAM and an increase in SAH concentration. This being different than the situation where methyl groups are limited resulting in decreased SAM and increased SAH (Wainfan and Poirier, 1992).

SAH is then hydrolyzed to homocysteine by Ahcy; mRNA expression of Ahcy is increased 3.8-fold in the dwarf mouse. Injection of GH to rats had no effect on activity of liver Ahcy (Finkelstein and Harris, 1973). Because the equilibrium of this reaction favors the production of SAH from homocysteine (Finkelstein, 2000), SAH will accumulate in situations where homocysteine accumulates. Although liver homocysteine was not measured, it most likely does not accumulate for several reasons: (1) we found that plasma homocysteine is significantly decreased in the Ames dwarf mouse (Uthus and Brown-Borg, unpublished observations), and (2) the activities of Cbs and Cth are both increased (Uthus and Brown-Borg, 2003). The increased tissue SAH seen in the Ames dwarf most likely reflects the increased synthesis of SAM from methionine and concomitant increase in transmethylation.

Homocysteine is at a crucial branch point in methionine metabolism. Here the backbone of methionine can be recycled by methylation of homocysteine to reform methionine. There are two reactions, one catalyzed by Bhmt and the other by Mtr, in which homocysteine can be remethylated. Alternatively, the backbone can be lost by entering the transsulfuration pathway where the fist reaction, catalyzed by Cbs, is irreversible. Although mRNA for Bhmt is increased in the dwarf mouse, the enzyme activity is not significantly different from the wild type mouse. Both Mtr mRNA and enzyme activity are decreased in the dwarf mouse. Based on this, and because the activities of Cbs and Cth are elevated (Uthus and Brown-Borg, 2003) in the Ames mouse, and because of the isotope data, it can be seen that homocysteine is readily shunted to transsulfuration.

Recent studies on purified Mtr and Cbs have shown reciprocal sensitivity of these two enzymes to oxidative conditions; Mtr is reduced (Chen et al., 1995) and Cbs is increased (Taoka et al., 1998) under oxidizing conditions. Thus, redox changes may serve a regulatory role such that oxidative stress would increase the flux of methionine through the transsulfuration pathway ultimately increasing the concentration of GSH (Mosharov et al., 2000). Mosharov et al. (2000) suggest that a 2–3-fold enhancement of methionine (via homocysteine) flux through transsulfuration in response to oxidative stress could be physiologically significant. The activity and/or mRNA of Cbs and Mtr in the Ames dwarf mimic these changes seen in oxidative stress [previously we showed that the specific activity of liver Cbs increased 50% and that of Mtr tended ($p < 0.06$) to be decreased in the dwarf mouse; Uthus and Brown-Borg, 2003]. However, the Ames dwarf is not under an oxidative stress. Thus, the increase in Cbs and the decrease in Mtr can be viewed as being part of a heightened oxidative defense system. Mtr mRNA was decreased, however, that of Cbs was unaffected in the dwarf mouse. Cth mRNA tended to be increased in dwarf liver compared to wild type. Cth enzyme activity was found to be elevated 83% in dwarf liver (Uthus and Brown-Borg, 2003). Mosharov et al. (2000) predicted that deficiency of this enzyme would affect the GSH pool only in tissues where the transsulfuration pathway operates (as it does in liver, kidney, and brain; Finkelstein, 1990, 2000; Awata et al., 1995).

One fundamental effect of the increased flux of methionine through transsulfuration is an increase in GSH. The Ames dwarf mouse has significantly increased hepatic GSH and we have shown that GSH utilization is decreased by GH administration to the dwarf mice (Brown-Borg et al., 2005). The markedly altered methionine metabolism in the Ames dwarf may play a significant role in their metabolism of GSH. Transsulfuration provides the direct link between methionine (via homocysteine) and GSH, the major redox buffer in mammalian cells. Therefore, the lack of GH in dwarf mice results in higher overall levels of tissue glutathione *S*-transferase (Brown-Borg et al., 2005) and GSH, thus enhancing the detoxification ability and providing cellular protection and resistance to toxic/oxidative challenges.

These results support the hypothesis that heightened antioxidative defensives may play a role in the life span extension (Brown-Borg et al., 1999; Brown-Borg and Rakoczy, 2000) and that the Ames dwarf mouse may be more resistant to oxidative stress because of the relatively large pool of total GSH due to altered activities or levels of GSH metabolites or components further upstream (Brown-Borg et al., 2005). Our results suggest that the mechanism for the altered GSH metabolism observed in dwarf mice may be the result of the distinctive methionine metabolism and that this distinctive methionine metabolism may be the result of lack of GH.

Acknowledgments

The authors are indebted to Kim Baurichter, Sharlene Rakoczy, and Tom Zimmerman for their excellent technical assistance.

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